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Review

Streptomyces inside-out: a new perspective on the bacteria that provide us with antibiotics

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Many of the antibiotics used today are made by a group of bacteria called *Streptomyces*. Streptomycetes evolved about 450 million years ago as branched filamentous organisms adapted to the utilization of plant remains. They reproduce by sending up specialized aerial branches, which form spores. Aerial growth is parasitic on the primary colony, which is digested and reused for aerial growth. The reproductive phase is coordinated with the secretion of antibiotics, which may protect the colony against invading bacteria during aerial growth. A clue to the integration of antibiotic production and aerial growth is provided by *bldA* mutants, which are defective in both processes. These mutants lack the ability to translate a particularly rare codon, UUA, in the genetic code. The UUA codon (TTA in DNA) is present in several regulatory genes that control sets of antibiotic production genes, and in one, *bldH* that controls aerial mycelium formation. The regulatory genes for antibiotic production are all involved in self-reinforcing regulatory systems that potentially amplify the regulatory significance of small changes in the efficiency of translation of UUA codons. One of the regulatory targets of *bldH* is an extracellular protease inhibitor protein that is likely to delay the digestion of the primary biomass until the colony is ready for aerial growth. The use of the UUA codon to orchestrate different aspects of extracellular biology appeared very early in *Streptomyces* evolution.

Keywords: *Streptomyces coelicolor*; *bldA*; codon usage; protease inhibitor; evolution of *Streptomyces*; tRNA

1. INTRODUCTION

Streptomycetes are the most important source of antibiotics for medical, veterinary and agricultural use. They belong to a class of bacteria of considerable interest to human welfare that are known as actinomycetes. This name, meaning 'ray fungi', was conferred because the first known example grew as fungus-like branching filaments, but actinomycetes are now known to encompass various forms. The simplest are unicellular spheres and rods, among which are the corynebacteria, including the agent of diphtheria as well as more benign industrial species used to make amino acids for food supplementation. Other actinomycetes are somewhat filamentous, including the mycobacteria that cause tuberculosis and leprosy. Members of the genus *Streptomyces* are the most complex: they grow as a mycelium of branching hyphal filaments, and reproduce in a mould-like manner by sending up aerial branches that turn into chains of spores. The coordination of this morphological development with both antibiotic production and other aspects of extracellular biology is the subject of this article.

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Based on the 2005 Leeuwenhoek lecture.

2. A BRIEF HISTORY OF *STREPTOMYCES* AND ANTIBIOTICS

Bacteria, the first form of cellular life on Earth, already existed some 3.5 billion years ago, when the Earth itself was about 1 billion years old. As a result of the development of photosynthesis by early bacteria, free oxygen appeared in the atmosphere about 2 billion years ago. This created new possibilities for living organisms to obtain energy. An explosion of bacterial diversity followed, and at that time a line of descent leading to present-day actinomycetes was established; but the first true streptomycete did not appear until about 450 million years ago, eventually giving rise to all today's *Streptomyces* species. The stimulus for this development, which would eventually prove momentous for human welfare, was probably the colonization of the land by green plants, about 550 million years ago (Embley & Stackebrandt 1994). There would have been powerful natural selection for organisms that could digest the tough plant materials and profit from the nutrients locked up in them. Micro-organisms such as streptomycetes and fungal moulds that grew as a mycelium of branching hyphal filaments were among those that appeared in response to this opportunity. Mycelial organisms could attach to, penetrate, and feed on dead plant tissues, helped by the secretion of powerful enzymes such as cellulases and xylanases. (It seems that streptomycetes also soon acquired the

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Table 1. Medical importance of some antibiotics from streptomycetes and their close relatives.

target disease or organism	antibiotic	producing organism
typhoid	chloramphenicol	*Streptomyces venezuelae*
TB and leprosy	rifampicin	*Amycolatopsis* (formerly *Streptomyces*) *mediterranei*
methicillin-resistant *Staphylococcus aureus* (MRSA)	vancomycin	*Amycolatopsis* (formerly *Streptomyces*) *orientalis*
river blindness	avermectin	*Streptomyces avermitilis*
cancer	daunomycin	*Streptomyces coeruleorubidus*
pathogens with transmissible penicillin resistance	clavulanic acid	*Streptomyces clavuligerus*

ability to attack their fungal competitors, since all streptomycetes are equipped with a battery of secreted chitinase enzymes that allow them to break down fungal cell walls.) To aid dispersal, some form of hyphal fragmentation must have coevolved with the coherent, fixed mycelial growth form, and this was presumably the starting point for the evolution of spores.

The photosynthetic activity of land plants caused the level of oxygen in the atmosphere to rise sharply from about 2%, stabilizing at its present-day level of 20% by about 300 million years ago. The increasing oxygenation of the atmosphere permitted the development of animals such as earthworms and arthropods. Selection for survival in the hostile guts of earthworms and arthropods may have been important in the evolution of the tough spores of streptomycetes. Perhaps the ability of streptomycetes to sporulate in the air, rather than directly converting their vegetative mycelium into spores, was an adaptation that made the spores more accessible to arthropods grazing on the microbes at the surfaces of plant remains. This would then have provided free transport to more remote locations.

Mammals did not emerge for about 200 million more years, and the first humans did not appear until about a million years ago. The first human to become aware of the existence of bacteria was Antonie van Leeuwenhoek, who observed unicellular bacteria in 1684, using a small spherical lens that he ground himself. Even then, it took nearly 200 more years before microbiology advanced further, with pioneers such as Louis Pasteur, Robert Koch and Ferdinand Cohn. Although Cohn was the first to describe what was undoubtedly a strain of *Streptomyces*, this name was eventually conferred by Selman Waksman in 1942. Waksman's interest in streptomycetes at that time was bound up with his epoch-making discovery that they make antibiotics (work for which he received a Nobel Prize). The first commercial/medical use of a *Streptomyces* antibiotic, the treatment of tuberculosis by streptomycin, was in the mid-1940s.

There followed a rich period in which many useful antibiotics were isolated from streptomycetes and similar actinomycetes. Table 1 gives an indication of the effects that these compounds have on some deadly diseases, and also makes the point that different streptomycetes make different antibiotics. The rate of discovery has tailed off since 1960, for reasons that are more commercial than biological—but there is little doubt that the known *Streptomyces* antibiotics are the tip of an iceberg.

In this section, three special features of *Streptomyces* biology have been emphasized: the complex life cycle, culminating in the formation of a sporulating aerial mycelium; the production of antibiotics; and the production of extracellular enzymes for the solubilization of plant-derived polymers. All three are typically manifested late in the life of a colony. This late timing can be rationalized as follows. The germination of a spore occurs in response to soluble nutrients, and early rapid mycelial growth exploits these soluble nutrients as 'easy meat'. Only when these nutrients have been used up does it become advantageous to export enzymes for the degradation of insoluble nutrients. By then, the greater *Streptomyces* biomass means that each individual hyphal compartment needs to secrete only a small amount of enzyme for the local concentration to reach effective levels. It makes some sense if antibiotics are secreted at the same time, because this protects the solubilized plant materials against other micro-organisms that might swim in and compete with the mycelium. This could even be considered as an ambush strategy, the would-be invaders constituting additional potential nutrition: a notion called 'fatal attraction' by Shi & Zussman (1993). Around this time, as the readily assimilable nutrients begin to run out, aerial mycelium forms. Aerial growth is at least partially parasitic on the mycelium from which it emerges, some of which breaks down to provide nutrition to the aerial hyphae (Wildermuth 1970). The antibiotics probably also protect these nutrients against invaders (figure 1; Chater & Merrick 1979).

Recently, great advances in our knowledge of the entire genetic composition of a couple of streptomycetes (Bentley *et al*. 2002; Ikeda *et al*. 2003) have helped to clarify some aspects of the linked genetic control of sporulation and antibiotic production. This is the focus of the remainder of this article.

3. *STREPTOMYCES COELICOLOR* GENETICS AND THE DISCOVERY OF A KEY TO THE SPECIAL BIOLOGY OF STREPTOMYCETES

As the first *Streptomyces* antibiotics were gaining medical use in the 1940s, other epoch-making discoveries involving bacteria were taking place (Brock 1990). Joshua Lederberg showed that bacteria, represented by *Escherichia coli*, could exchange genetic material. Avery & McCleod proved that the genetic material was DNA. At Cambridge, Watson & Crick established modern molecular biology with their brilliant solution of the structure and core function of DNA.

Against this background, a graduate student at Cambridge began work on the genetics and molecular biology of antibiotic-producing streptomycetes. David

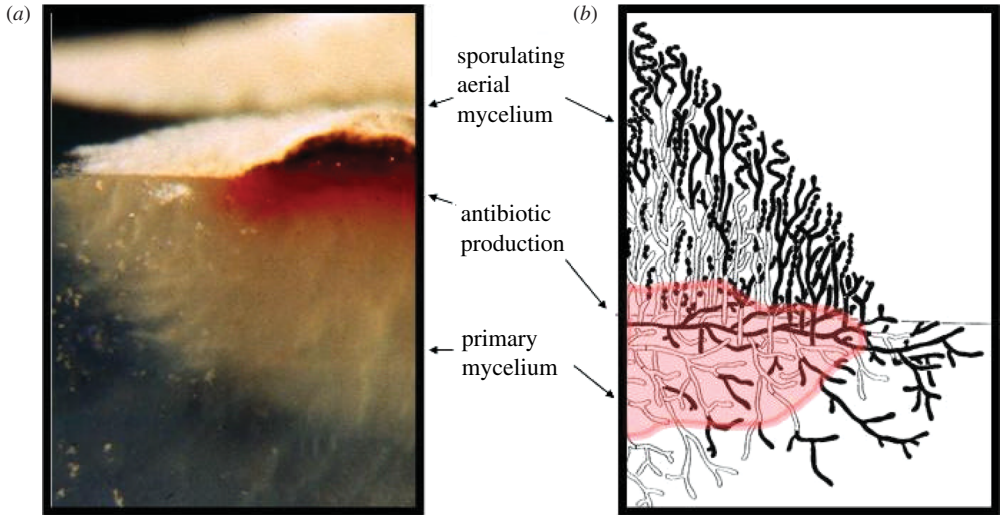

Figure 1. (*a*) Vertical sections through a *Streptomyces* colony. Photograph of colony growing on agar (courtesy of Jamie Ryding). (*b*) Diagram indicating how antibiotic production in the lower part of the colony can protect the nutrients released from dead cells (white) so that they can support aerial growth and sporulation. Living cells are shown as black (after Wildermuth 1970).

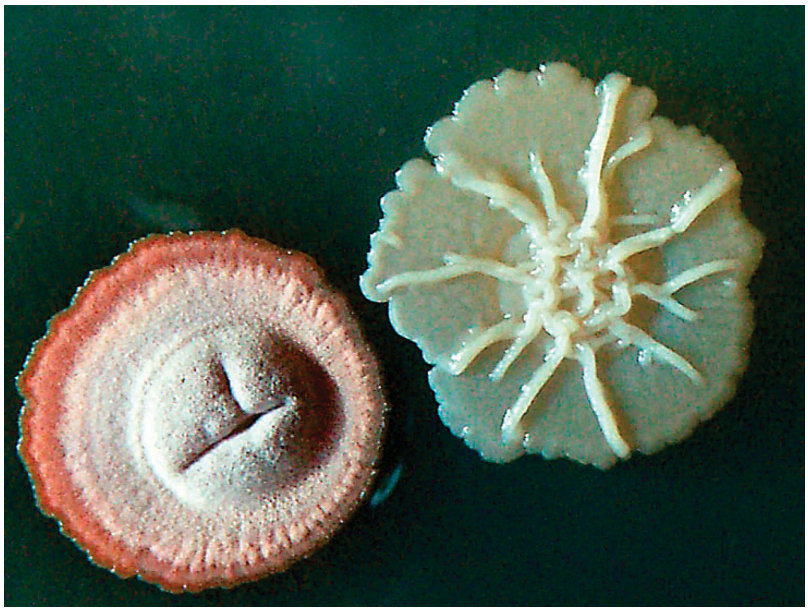

Figure 2. The effect of a mutation in the *bldA* gene. The colony on the left is normal, with a powdery sporulating surface and areas coloured by pigmented antibiotics. The colony on the right lacks the *bldA* gene, has a wrinkled, shiny, non-sporulating surface, and makes no pigments (photograph by Tobias Kieser).

Hopwood's chosen organism, *Streptomyces coelicolor*, became the model species for such work internationally after the first coherent map of various genes on the *S. coelicolor* chromosome was published (Hopwood 1967). That article showed an example of the genetic mapping of a previously unknown mutation, then called S48. The mutation was extraordinary, because it affected more than one function: both the blue pigment responsible for the name *coelicolor* ('sky-blue') and the ability to produce aerial mycelium were lost. In the genetic cross that Hopwood described, the progeny of the mating were all either blue and morphologically normal, or unpigmented and devoid of aerial mycelium. This complete linkage of the two characteristics formally showed that the same mutation was responsible for both (as we see later, other biological properties of the mutant were also changed). Merrick (1976) found that other similar mutations mapped in the same place, and named the gene concerned *bldA* (because of the 'bald' appearance of the colonies; figure 2).

The next phase in *Streptomyces* genetics was the development of methods for the isolation of particular genes. One of these took advantage of a virus of *Streptomyces* (bacteriophage phiC31) that had been discovered in Moscow (Lomovskaya *et al.* 1972). A way was found to insert random pieces of *Streptomyces* DNA into specially engineered derivatives of phiC31, to give a library of cloned genes. One of the clones could restore aerial growth and pigment production to a *bldA* mutant, and thus identified the *bldA* gene (Piret & Chater 1985). Most unusually, this gene encoded a transfer RNA (Lawlor *et al.* 1987). Such tRNAs, small RNA molecules less than 100 bases long, serve to translate the base triplets of the genetic code into one of the 20 amino acids in proteins (figure 3). The specific

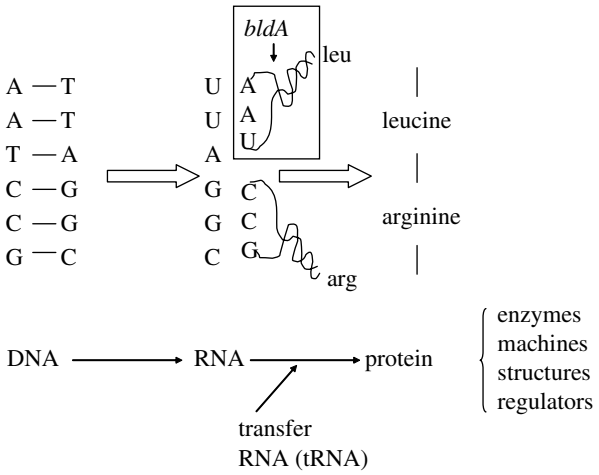

Figure 3. The role of the tRNA encoded by *bldA*. The diagram shows the transcription of a short stretch of DNA sequence containing TTA and GGC codons into messenger RNA containing UUA and GGC codons, and their translation, via the *bldA* tRNA and another tRNA, into leucine and arginine residues in a stretch of protein.

job of the *bldA* tRNA is to add a leucine residue to growing proteins at positions corresponding to the codon UUA, which in turn corresponds to TTA in the DNA from which the messenger RNA has been transcribed.

UUA is one of six different codons for leucine. It is special in two respects. First, in contrast to some other codons, no other tRNA can translate it efficiently (Crick 1966), so UUA codons depend strongly on the *bldA* tRNA for translation (Leskiw *et al.* 1991). Second, *Streptomyces* DNA is unusually rich in G and C bases, and correspondingly poor in T and A bases, so TTA codons are rare in *Streptomyces* genes. In fact, of the 7825 genes revealed by the sequencing of the genome of *S. coelicolor* (Bentley *et al.* 2002), only 145 contain a TTA codon. Detailed studies were, therefore, undertaken to find out if any of these TTA-containing genes hold a key to the processes of antibiotic production and colony differentiation.

4. TTA CODONS IN REGULATORY GENES FOR ANTIBIOTIC SYNTHESIS

The protein composition of cells determines their structure and function, including their ability to make all the small molecules that contribute to their evolutionary fitness. In turn, protein composition is determined by gene sequences. The 7825 genes revealed by the genome sequence of *S. coelicolor* showed that the organism was likely to contain thousands of proteins. Experimentally, many of the proteins could be directly displayed as spots by two-dimensional gel electrophoresis (see figure 4 for an example and explanation). To show which gene any protein spot corresponded to, individual spots were then cut out and processed to generate a molecular fingerprint. This was done by treating them with a protease such as trypsin, an enzyme that cuts peptide bonds in proteins at positions immediately following either of the two amino acids arginine and lysine. This process generates small protein fragments that are characteristic of a particular protein and are readily predictable for any gene whose DNA sequence is known. Thus, when the masses of the protein fragments were revealed in a mass spectrometer, they could be compared by computer to all the fragments predicted for all the genes in the genome, to reveal the protein : gene correspondence (Hesketh *et al.* 2002).This technology has recently been used to compare a *bldA* mutant with its wild-type parent (Kim *et al.* 2005*a*,*b*).

Considering the startling effects of *bldA* mutations on *Streptomyces* colonies, it was curious to find that the global patterns of protein spots seen on two-dimensional gels were largely unaffected, many of the changes being in the proteins concerned with antibiotic production: proteins involved in the production of seven different antibiotics or antibiotic-like molecules were altered in abundance in the mutant (A. Hesketh & K. F. Chater 2005, unpublished work). This was consistent with earlier evidence that *bldA* mutants were defective in the production of several antibiotics (Merrick 1976; Champness 1988).

Some of these effects of the *bldA* mutation could be tied down to the presence of TTA codons in relevant genes. For the *act* and *red* genes the protein products of these genes were regulatory in nature (Fernandez-Moreno *et al.* 1991; Passantino *et al.* 1991; White & Bibb 1997; Guthrie *et al.* 1998), while in the case of a gene set for an unknown oligosaccharide end product, the TTA-containing genes encoded enzymes in the biosynthetic process. The effects of *bldA* on the other four gene sets remain unexplained.

There is circumstantial evidence that the TTA-containing regulatory genes *actII-4* and *redZ* are involved in systems that incorporate self-reinforcing positive feedback loops, albeit of an as yet uncharacterized kind, since even a single extra copy of *actII-4* or of the target of *redZ* (another regulatory gene, *redD*) causes a disproportionate increase in production of the relevant antibiotic (Narva & Feitelson 1990; Passantino *et al.* 1991; figure 5). Thus, *bldA* intervenes at a particularly sensitive regulatory point in the *act* and *red* systems, such that any change of *bldA* activity is expected to be strongly amplified. This theme was strikingly illustrated in work on another set of genes for antibiotic synthesis from *S. coelicolor*. These genes, for methylenomycin synthesis, are located on an extrachromosomal element instead of the chromosome. Merrick (1976) had shown that *bldA* mutants could not make methylenomycin, so it was interesting when DNA sequencing revealed TTA codons in genes *mmfL* and *mmyB* in the gene cluster for methylenomycin production (Bentley *et al.* 2004). To investigate the significance of these TTA codons, each was changed to an alternative leucine codon (O'Rourke *et al.* in preparation). The codon change in *mmfL* allowed the *bldA* mutant to produce a chemically uncharacterized extracellular substance that acted as a signal for methylenomycin production. The other codon change, in the *mmyB* regulatory gene, permitted the *bldA* mutant to respond to this signal by switching on the genes for methylenomycin synthesis. When both codon changes were made, methylenomycin production became independent of *bldA*.

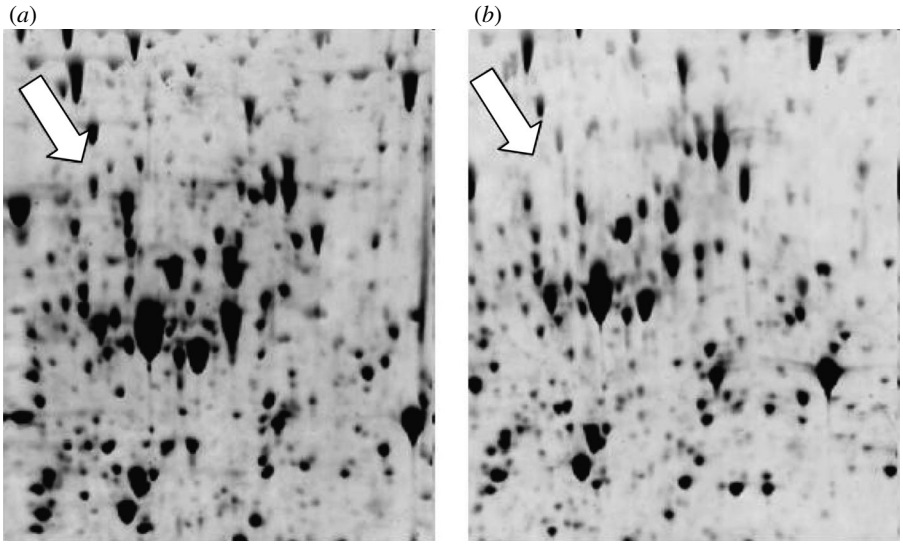

Figure 4. Comparison of a (*a*) *bldA* mutant with a (*b*) wild-type strain by two-dimensional electrophoresis. Proteins in cell extracts were first lined up according to their intrinsic charge by exposing them to an electric potential difference in a long, narrow matrix with a fixed gradient of pH. This matrix was then treated to eliminate the charge differences between proteins, and embedded at one side of a rectangular slab of polyacrylamide gel. A current was applied at right angles to the direction of the first separation, dragging the proteins through the gel. Small proteins pass through the structure of such gels more readily than larger ones, so the resulting gel, after suitable staining of the protein spots, provided a snapshot of the protein composition of the starting cellular material. The arrows indicate a protein spot absent from the mutant. Note that most spots are present in both strains, though the precise positions of spots change somewhat between gels. (Photograph courtesy of Andy Hesketh.)

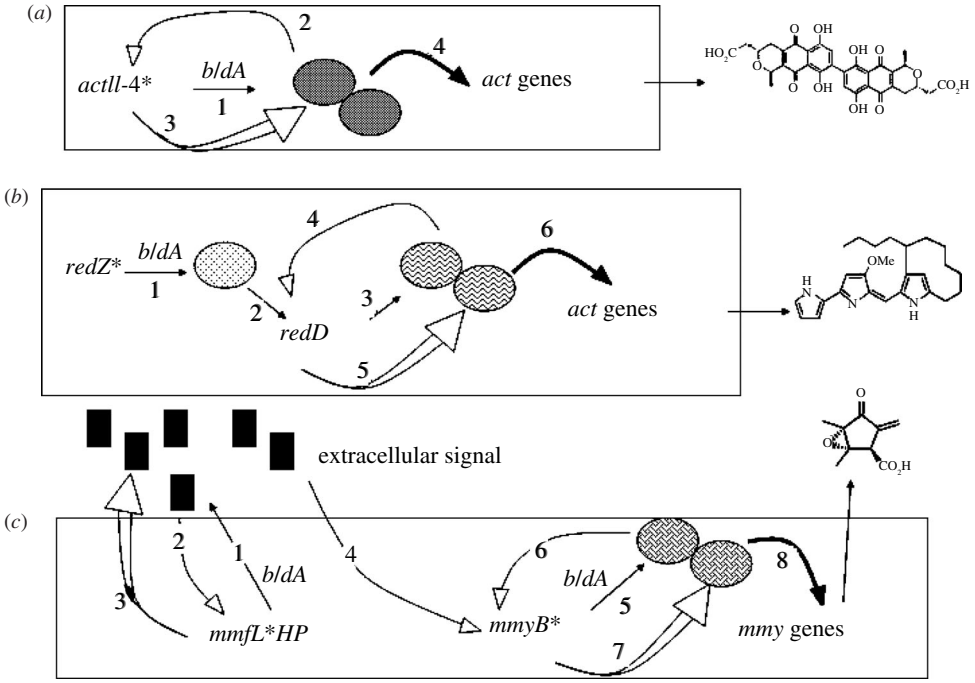

Figure 5. Role of *bldA* in autoregulatory circuits for antibiotic production. Three gene sets are illustrated. Shaded ovals represent regulatory proteins. The numbers indicate the sequence of events leading to expression of production genes for actinorhodin (*act*, *a*), undecylprodigiosin (*red*, *b*) and methylenomycin (*mmy*, *c*). In each case the first step involves a gene with a TTA codon (asterisks). The three systems differ in complexity from this point. In the simplest example, the TTA-containing gene (*actII-4*) encodes a regulatory protein that exerts some kind of positive feedback (*act*, steps 2 and 3; see text), and thereby accumulates to a level at which it can activate genes for biosynthesis of the end product (step 4). In the case of the TTA-containing *redZ*, another regulatory gene (*redD*) is activated (step 3), and this goes through an autoregulatory reinforcement, again uncharacterized (steps 4 and 5; see text), before activating the genes for synthesis of the end product (step 6). In the most complex case, expression of the TTA-containing *mmfL*, together with *mmfH* and *mmfP*, causes accumulation of a small extracellular signal molecule (step 1), which activates further synthesis of itself (steps 2 and 3) to a level that can activate another regulatory gene, *mmyB*, that also contains a TTA (step 4). If *bldA* tRNA is available, expression of *mmyB* can undergo another autoregulatory loop (steps 6 and 7) so that enough of the *mmyB* gene product accumulates to switch on the genes for synthesis of the final product, methylenomycin (step 8).

Both *mmfL* and *mmyB* are involved in their own positive feedback regulation, making the methylenomycin system a particularly striking example of how very big effects might be brought about by quite small changes in the availability of the *bldA* tRNA. Previous work had indeed established that the abundance of this tRNA relative to other tRNAs increases markedly late in growth, potentially providing an important signal for the onset of antibiotic production (Leskiw *et al.* 1993; Trepanier *et al.* 1997). There seems to be widespread use of *bldA*-dependent regulation of the synthesis of diverse antibiotics among other streptomycetes, since TTA codons can be found in the regulatory genes of half of 54 gene sets for antibiotic biosynthesis that have been sequenced in various laboratories (G. Chandra & K. F. C., unpublished work).

5. BROADER EFFECTS OF *bldA* ON EXTRACELLULAR BIOLOGY

In the two-dimensional gel experiments described earlier (e.g. figure 4), the cellular material used had been concentrated away from the original growth medium, and so would have been separated from any secreted proteins with biological functions outside of the cells. When two-dimensional gel electrophoresis was used to analyse the medium after the cellular material had been removed, about 600 protein spots appeared when the culture was fully grown, though there were no detectable extracellular proteins at earlier stages of growth (Kim *et al.* 2005*b*). Of these spots, 21 were changed in intensity in the *bldA* mutant, revealing new avenues to be explored in the *bldA* world, as is illustrated by the protein corresponding to gene SCO0762. This protein was found to be very similar in its amino acid sequence to proteins that, in other streptomycetes, prevent certain proteases from carrying out their normal function of digesting other proteins. Indeed, an inhibitory activity of this kind (called STI, for small trypsin inhibitor) was found to be present in cultures of the wild-type *S. coelicolor*, and absent from the *bldA* mutant.

Previously, one of these protease inhibitors had been implicated in sporulation of *Streptomyces exfoliatus* (Kim & Lee 1995). Its purpose appears to be to hold an extracellular trypsin-like protease inactive until the culture is fully grown and ready to sporulate, at which time another protease is secreted that targets the STI protein. Degradation of the STI releases the trypsin-like protease, which then digests proteins of the old mycelium so that they can be reused as nutrients to support the growth of the reproductive aerial mycelium. Work to find out whether this cascade also functions in *S. coelicolor* is still in progress; but a candidate for the protease that might degrade STI has been detected in wild-type culture fluids, and is greatly reduced in amount in a *bldA* mutant (D. W. Kim & K. J. Lee, personal communication).

6. HOW *bldA* CONTROLS THE LEVELS OF STI

Neither the STI gene nor any gene near it on the chromosome contains a TTA codon, so what makes it *bldA*-dependent? The starting point for answering this question was some observations made by Willey *et al.* (1993). In the course of studying several different kinds of *bld* mutants, including *bldA*, it was found that the growth of pairs of mutants close together on Petri dishes often resulted in the growth of aerial mycelium on one of the mutants. This effect suggested that the secretion of some material by one mutant could stimulate the other mutant to develop. A sequence of several extracellular signals was deduced from the complete set of interactions (figure 6). The place of a *bldA* mutant in this cascade was the same as that of a *bldH* mutant.

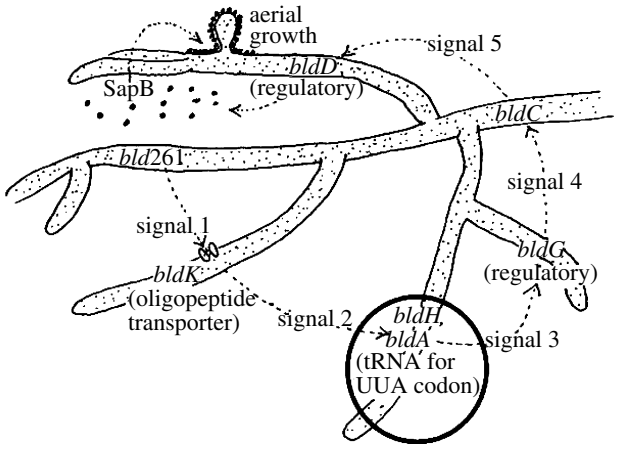

Figure 6. Mutants in the *bldA* and *bldH* genes behave similarly in a cascade of extracellular signals that leads to aerial growth. The scheme is based on results of Willey *et al.* (1993), and was adapted from Chater (1998).

This similarity between the two mutants was explained when *bldH* was found to contain a TTA codon (Nguyen *et al.* 2003; Takano *et al.* 2003). When this codon was changed to an alternative leucine codon, a *bldA* mutant regained the ability to make sporulating aerial mycelium. Could the STI deficiency of the *bldA* mutant be attributed to an involvement of *bldH* in the expression of the STI gene? Support for this came from work on the *Streptomyces griseus bldH* gene (called *adpA* in *S. griseus*). The *adpA* gene encodes a key regulator (AdpA) of multiple functions. To carry out this regulation, AdpA recognizes a short DNA sequence next to its target genes (Ohnishi *et al.* 2005). In *S. coelicolor*, two likely AdpA recognition sequences could be found next to the STI gene. It was, therefore, gratifying to find that the expression of the STI gene was entirely eliminated by mutation of *bldH* (Kato *et al.* 2005*b*; Kim *et al.* 2005*b*). Further, *S. griseus* produces several proteases that are sensitive to STI-like proteins, and are encoded by AdpA-dependent genes, and at least one of these contributes to aerial development of *S. griseus*. This provides further evidence that the extracellular protease cascade is controlled by the *bldA*/AdpA system (Kato *et al.* 2002, 2005*a*).

7. CONCLUSIONS: THE PERVASIVE EFFECTS OF *bldA* ON EXTRACELLULAR BIOLOGY

Starting from a consideration of the evolution and characteristics of streptomycetes, it appears that some of the most characteristic special features of these bacteria involve the production of extracellular molecules (including proteins, antibiotics and signal

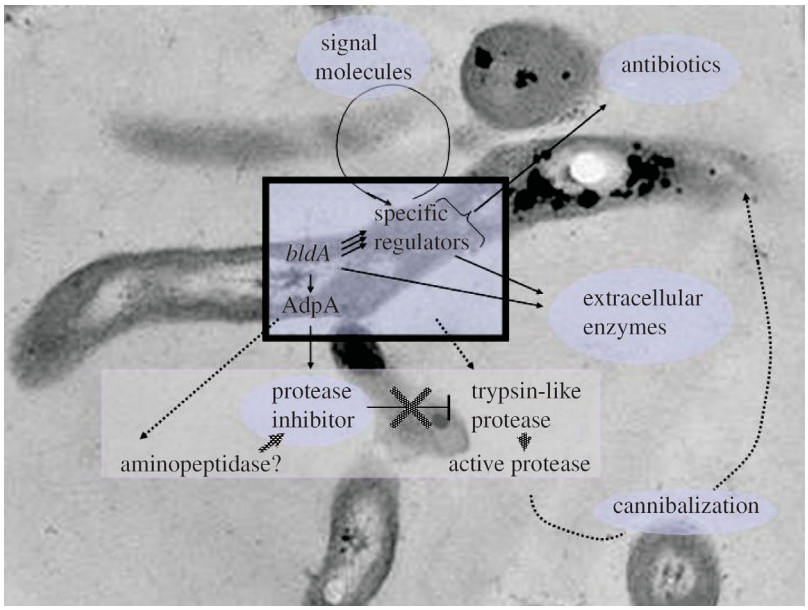

Figure 7. The presence of TTA codons in key regulatory genes influences several aspects of the extracellular biology of *S. coelicolor*. The rectangular box encloses processes taking place inside a hyphal compartment (the underlying image is an electron micrograph of a thin section through hyphae of *S. coelicolor*). The extracellular cascade thought to activate cannibalization by a trypsin-like protease is indicated by shaded arrows and a shaded cross. Dotted lines indicate processes that have been demonstrated in another species, but have yet to be proven for *S. coelicolor*.

molecules) at about the time when the main growth period is ending. By studying a mutant with multiple defects in these processes, a picture has emerged in which the availability of a particular leucyl-tRNA to translate UUA codons in the mRNA of various regulatory proteins determines the abundance of these proteins.

The proteins themselves have as their ultimate targets the genes for various processes, including the synthesis of different antibiotics and extracellular enzymes, and the development of the sporulating aerial mycelium that will ensure the continued propagation and dispersal of the species (figure 7). These regulatory proteins often also take part in self-amplifying regulatory circuits. This gives considerable potential for amplifying the signalling significance of small changes in UUA-translating ability. In some cases, the processes that the regulatory proteins control are also potentially self-amplifying. This is the case for the signalling molecule for methylenomycin synthesis, and potentially also for the extracellular protease cascade that STI is involved in. However, many questions are unanswered, including not just how the level of UUA-translating capacity is controlled, but whether UUA translatability is a genuine regulatory device. Nevertheless, the fact that a TTA codon is present in *adpA* genes from several streptomycetes whose last common ancestor probably lived about 250 million years ago (A. Ward personal communication) makes it likely that this is an ancient and, therefore, important trait; and the conservation among streptomycetes of a handful of other TTA-containing genes may provide a way into some of the most fundamental aspects of the biology and evolution of these complex and important microbes.

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REFERENCES

Bentley, S. D. *et al*. 2002 Complete genome sequence of the model actinomycete *Streptomyces coelicolor* A3(2). *Nature* **417**, 141–147. (doi:10.1038/417141a)

Bentley, S. D. *et al*. 2004 SCP1, a 356 023 bp linear plasmid adapted to the ecology and developmental biology of its host, *Streptomyces coelicolor* A3(2). *Mol. Microbiol.* **51**, 1615–1628. (doi:10.1111/j.1365-2958.2003.03949.x)

Brock, T. D. 1990 *The emergence of bacterial genetics*. Cold Spring Harbor, New York: Cold Spring Harbor Press.

Champness, W. 1988 New loci required for *Streptomyces coelicolor* morphological and physiological differentiation. *J. Bacteriol.* **170**, 1168–1174.

Chater, K. F. 1998 Taking a genetic scalpel to the *Streptomyces* colony. *Microbiology* **144**, 1465–1478.

Chater, K. F. & Merrick, M. J. 1979 *Streptomyces*. In *Developmental Biology of Prokaryotes* (ed. J. H. Parish), pp. 93–114. Oxford, UK: Blackwell.

Crick, F. 1966 Codon–anticodon pairing: the wobble hypothesis. *J. Mol. Biol.* **19**, 548–555.

Embley, T. & Stackebrandt, E. 1994 The molecular phylogeny and systematics of the actinomycetes. *Annu. Rev. Microbiol.* **48**, 257–289.

Fernandez-Moreno, M., Caballero, J. L., Hopwood, D. A. & Malpartida, F. 1991 The act cluster contains regulatory and antibiotic export genes, direct targets for translational control by the *bldA* tRNA gene of *Streptomyces*. *Cell* **66**, 769–780. (doi:10.1016/0092-8674(91)90120-N)

Guthrie, E. P., Flaxman, C. S., White, J., Hodgson, D. A., Bibb, M. J. & Chater, K. F. 1998 A response-regulator-like activator of antibiotic synthesis from *Streptomyces coelicolor* A3(2) with an amino-terminal domain that lacks a phosphorylation pocket. *Microbiology* **144**, 2007–2007.

Hesketh, A. R., Chandra, G., Shaw, A. D., Rowland, J. J., Kell, D. B., Bibb, M. J. & Chater, K. F. 2002 Primary and secondary metabolism, and post-translational protein

modifications, as portrayed by proteomic analysis of *Streptomyces coelicolor*. *Mol. Microbiol.* **46**, 917–932. (doi:10.1046/j.1365-2958.2002.03219.x)

Hopwood, D. 1967 Genetic analysis and genome structure in *Streptomyces coelicolor*. *Bacteriol. Rev.* **31**, 373–403.

Ikeda, H., Ishikawa, J., Hanamoto, A., Shinose, M., Kikuchi, H., Shiba, T., Sakaki, Y., Hattori, M. & Omura, S. 2003 Complete genome sequence and comparative analysis of the industrial micro-organism *Streptomyces avermitilis*. *Nat. Biotechnol.* **21**, 526–531. (doi:10.1038/nbt820)

Kato, J., Suzuki, A., Yamazaki, H., Ohnishi, Y. & Horinouchi, S. 2002 Control by A-factor of a metalloendopeptidase gene involved in aerial mycelium formation in *Streptomyces griseus*. *J. Bacteriol.* **184**, 6016–6025. (doi:10.1128/JB.184.21.6016-6025.2002)

Kato, J., Chi, W. J., Ohnishi, Y., Hong, S. K. & Horinouchi, S. 2005*a* Transcriptional control by A-factor of two trypsin genes in *Streptomyces griseus*. *J. Bacteriol.* **187**, 286–295. (doi:10.1128/JB.187.1.286-295.2005)

Kato, J., Hirano, S., Ohnishi, Y. & Horinouchi, S. 2005*b* The *Streptomyces* subtilisin inhibitor (SSI) gene in *Streptomyces coelicolor* A3(2). *Biosci. Biotechnol. Biochem.* **69**, 1624–1629. (doi:10.1271/bbb.69.1624)

Kim, I. & Lee, K. J. 1995 Physiological roles of leupeptin and extracellular proteases in mycelium development of *Streptomyces exfoliatus* SMF13. *Microbiology* **141**, 1017–1025.

Kim, D. W., Chater, K. F., Lee, K. J. & Hesketh, A. 2005*a* Effects of growth phase and the developmentally significant *bldA*-specified tRNA on the membrane-associated proteome of *Streptomyces coelicolor*. *Microbiology* **151**, 2707–2720. (doi:10.1099/mic.0.28000-0)

Kim, D. W., Chater, K., Lee, K. J. & Hesketh, A. 2005*b* Changes in the extracellular proteome caused by the absence of the *bldA* gene product, a developmentally significant tRNA, reveal a new target for the pleiotropic regulator AdpA in *Streptomyces coelicolor*. *J. Bacteriol.* **187**, 2957–2966. (doi:10.1128/JB.187.9.2957-2966.2005)

Lawlor, E. J., Baylis, H. A. & Chater, K. F. 1987 Pleiotropic morphological and antibiotic deficiencies result from mutations in a gene encoding a transfer RNA-like product in *Streptomyces coelicolor* A3(2). *Genes Dev.* **1**, 1305–1310.

Leskiw, B. K., Lawlor, E. J., Fernandez-Abalos, J. M. & Chater, K. F. 1991 TTA codons in some genes prevent their expression in a class of developmental, antibiotic-negative, *Streptomyces mutants*. *Proc. Natl Acad. Sci. USA* **88**, 2461–2465.

Leskiw, B. K., Mah, R., Lawlor, E. J. & Chater, K. F. 1993 Accumulation of *bldA*-specified transfer-RNA is temporally regulated in *Streptomyces coelicolor* A3(2). *J. Bacteriol.* **175**, 1995–2005.

Lomovskaya, N., Mkrtumian, N. M., Gostimskaya, N. L. & Danilenko, V. N. 1972 Characterization of temperate actinophage phi C31 isolated from *Streptomyces coelicolor*. *J. Virol.* **9**, 258–262.

Merrick, M. 1976 A morphological and genetic mapping study of bald colony mutants of *Streptomyces coelicolor*. *J. Gen. Microbiol.* **96**, 299–315.

Narva, K. & Feitelson, J. S. 1990 Nucleotide sequence and transcriptional analysis of the *redD* locus of *Streptomyces coelicolor*A3(2). *J. Bacteriol.* **172**, 326–333.

Nguyen, K. T., Tenor, J., Stettler, H., Nguyen, L. T., Nguyen, L. D. & Thompson, C. J. 2003 Colonial differentiation in *Streptomyces coelicolor* depends on translation of a specific codon within the *adpA* gene. *J. Bacteriol.* **185**, 7291–7296. (doi:10.1128/JB.185.24.7291-7296.2003)

Ohnishi, Y., Yamazaki, H., Kato, J. Y., Tomono, J. Y. & Horinouchi, S. 2005 AdpA, a central transcriptional regulator in the A-factor regulatory cascade that leads to morphological development and secondary metabolism in *Streptomyces griseus*. *Biosci. Biotechnol. Biochem.* **69**, 431–439. (doi:10.1271/bbb.69.431)

O'Rourke, S., Wietzorrek, A. & Chater, K. F. In preparation. The biosynthesis of the plasmid-determined antibiotic methylenomycin is governed by a *bldA* tRNA-dependent extracellular signalling system.

Passantino, R., Puglia, A. M. & Chater, K. 1991 Additional copies of the *actII* regulatory gene induce actinorhodin production in pleiotropic *bld* mutants of *Streptomyces coelicolor* A3(2). *J. Gen. Microbiol.* **137**, 2059–2064.

Piret, J. M. & Chater, K. F. 1985 Phage-mediated cloning of *bldA*, a region involved in *Streptomyces coelicolor* morphological development, and its analysis by genetic complementation. *J. Bacteriol.* **163**, 965–972.

Shi, W. & Zusman, D. 1993 Fatal attraction. *Nature* **366**, 414–415. (doi:10.1038/366414a0)

Takano, E., Tao, M., Long, F., Bibb, M. J., Wang, L., Li, W., Buttner, M. J., Deng, Z. X. & Chater, K. F. 2003 A rare leucine codon in *adpA* is implicated in the morphological defect of *bldA* mutants of *Streptomyces coelicolor*. *Mol. Microbiol.* **50**, 475–486. (doi:10.1046/j.1365-2958.2003.03728.x)

Trepanier, N. K., Nguyen, G. D., Leedell, P. J. & Leskiw, B. K. 1997 Use of polymerase chain reaction to identify a leucyl tRNA in *Streptomyces coelicolor*. *Gene* **193**, 59–63. (doi:10.1016/S0378-1119(97)00077-2)

White, J. & Bibb, M. J. 1997 The *bldA*-dependence of undecylprodigiosin production in *Streptomyces coelicolor* A3(2) involves a pathway-specific regulatory cascade. *J. Bacteriol.* **179**, 627–633.

Wildermuth, H. 1970 Development and organization of the aerial mycelium in *Streptomyces coelicolor*. *J. Gen. Microbiol.* **60**, 43–50.

Willey, J., Schwedock, J. & Losick, R. 1993 Multiple extracellular signals govern the production of a morphogenetic protein involved in aerial mycelium formation by *Streptomyces coelicolor*. *Genes Dev.* **7**, 895–903.